

Applicant: Reiter, et al.
 U.S. Serial No.: 09/855,153
 Filed: May 14, 2001
 Page: 2

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 issued July 31, 2001, claiming the priority of provisional applications, U.S. Serial No. 60/228,816 filed March 10, 1997; U.S. Serial No. 60/071,141 filed January 12, 1998 and U.S. Serial No. 60/074,675 filed February 13, 1998. Further, this application claims the priority of U.S. Serial No. 09/564,329 filed May 3, 2000, which claims the benefit of the filing dates of U.S. Serial No. 60/113,230 filed December 21, 1998, U.S. Serial No. 60/120,536 filed February 17, 1999 and U.S. Serial No. 60/124,658 filed March 16, 1999. The contents of all the foregoing applications are incorporated by reference into the present application.

Please replace the paragraph at page 30, lines 17-26 with the following rewritten paragraph:

D2
~~The~~ amino acid sequence of PSCA presented herein may be used to select specific regions of the PSCA protein for generating antibodies. For example, hydrophobicity and hydrophilicity analyses of the PSCA amino acid sequence may be used to identify hydrophilic regions in the PSCA structure. Regions of the PSCA protein that show immunogenic structure, as well as other regions and domains, can readily be identified using various other methods known in the art, such as Chou-Fasman, Garnier-Robson, Kyte-Doolittle, Eisenberg, Karplus-Schultz or Jameson-Wolf analysis. Fragments containing these residues are particularly suited in generating specific classes of anti-PSCA antibodies. Particularly useful fragments include, but are not limited to, the sequences TARIRAVGLLTVISK (SEQ. ID NO.: 16) and SLNCVDDSDYVVGK (SEQ. ID NO.: 18).

Please replace the paragraph at page 38, lines 2-5 with the following rewritten paragraph:

D3
~~Fragments~~ of human PSCA that are particularly useful as selective hybridization probes or PCR primers can be readily identified from the entire PSCA sequence using art-known methods. One set of PCR primers that are useful for RT-PCR analysis comprise 5' - TGCTTGCCCTGTTGATGGCAG - (SEQ. ID NO.: 19) and 3' - CCAGAGCAGCAGGCCGAGTGCA - (SEQ. ID NO.: 20).

Applicant: Reiter, et al.
 U.S. Serial No.: 09/855,153
 Filed: May 14, 2001
 Page: 3

Please replace the paragraph at page 66, lines 16-22 with the following rewritten paragraph:

D4 -- In preferred embodiments, DNA fragments of 9kb, 6kb, 3kb, and 1kb derived from the 5' upstream region of the PSCA gene, as shown in Figure 42, were produced by techniques described herein. The 9kb PSCA upstream region (pEGFP—PSCA) is involved with gene regulatory activity and was deposited on May 17, 1999 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852 and has there been identified as follows ATCC No. PTA-80. The 9 kb fragment was obtained by amplification using a T7 primer and RlhPSCA3'-5 (5'-gggaattcgacagccttcagggtc-3') (SEQ ID NO. 21).

Please replace the paragraph at page 80, lines 15-29 with the following rewritten paragraph:

DS -- RDA, Northern Analysis and RT-PCR: Representational difference analysis of androgen dependent and independent LAPC-4 tumors was performed as previously described (Braun et al., 1995, Mol. Cell. Biol. 15: 4623-4630). Total RNA was isolated using Ultraspec^R RNA isolation systems (Biotecx, Houston, TX) according to the manufacturer's instructions. Northern filters were probed with a 660bp RDA fragment corresponding to the coding sequence and part of the 3' untranslated sequence of PSCA or a ~400bp fragment of PSA. The human multiple tissue blot was obtained from Clontech and probed as specified. For reverse transcriptase (RT)-PCR analysis, first strand cDNA was synthesized from total RNA using the GeneAmp RNA PCR core kit (Perkin Elmer-Roche, New Jersey). For RT-PCR of human PSCA transcripts, primers 5'-tgcttgccctgttgatggcag- and 3'-ccagagcagcaggccgagtgca- were used to amplify a ~320 bp fragment. Thermal cycling was performed by 25-25 cycles of 95° for 30 sec, 60° for 30sec and 72° for 1 min, followed by extension at 72° for 10 min. Primers for GAPDH (Clontech) were used as controls. For mouse PSCA, the primers used were 5' -

Applicant: Reiter, et al.
 U.S. Serial No.: 09/855,153
 Filed: May 14, 2001
 Page: 4

D5
 ttctcctgctggccacctac- and 3' -gcagctcatcccttcacaat- (SEQ ID NO. 19 and SEQ ID NO. 20 respectively). --

Please replace the paragraph at page 89, lines 7-17 with the following rewritten paragraph:

-- Generation and Production of Monoclonal Antibodies: BALB/c mice were immunized three times with a purified PSCA-glutathione S-transferase (GST) fusion protein containing PSCA amino acids 22-99 (FIG. 1B). Briefly, the PSCA coding sequence corresponding to amino acids 18 through 98 of the human PSCA amino acid sequence was PCR-amplified using the primer pair:

D6
 5'- GGAGAATTCATGGCACTGCCCTGCTGTGCTAC (SEQ ID NO. 22)

3'-GGAGAATTCCTAATGGGCCCCGCTGGCGTT (SEQ ID NO. 23)

The amplified PSCA sequence was cloned into pGEX-2T (Pharmacia), used to transform E. coli, and the fusion protein isolated. --

Please replace the paragraph at page 107, lines 2-26 with the following rewritten paragraph:

D7
 -- The reporter gene vectors are depicted in Figure 42 and were constructed as follows. The 14 kb Not I fragment was sub-cloned from the λ vector into a Bluescript KS vector (Stratagene), resulting in the pBSKS-PSCA (14kb) construct. The PSCA upstream sequence was subcloned from pBSKS-PSCA (14 kb) by PCR amplification using a primer corresponding to the T7 sequence contained within the Bluescript vector, and a primer corresponding to a sequence contained within PSCA exon 1 (primer H3hPSCA3'-5, the sequence of this primer is as follows: The sequence of H3hPSCA3'-5 is 5'-gggaagcttgacagccttcagggtc-3' (SEQ ID NO. 24). The primer corresponding to PSCA exon 1 contained an introduced HindIII sequence to allow further subcloning following PCR amplification. The resulting amplified fragment was digested with HindIII and was

Applicant: Reiter, et al.
 U.S. Serial No.: 09/855,153
 Filed: May 14, 2001
 Page: 5

D7
 subcloned into the pGL3-basic vector (Promega) to generate pGL3-PSCA (7 kb) which was used to generate a series of deletion reporter gene constructs containing varying lengths of PSCA upstream sequences operatively linked to the luciferase gene (Figure 42). The deleted portions of the PSCA upstream regions were obtained by subcloning restriction fragments from pGL3-PSCA (7 kb). The PSCA upstream region between -9 kb and -7 kb was subcloned from the pBSKS-PSCA (14 kb) construct, the Not I site was converted into a blunt end by Klenow and the fragment was cloned into the SacI/HindIII sites of pGL-PSCA (7 kb) in order to obtain the pGL3-PSCA (9 kb) construct. The reference to the sequences upstream of the PSCA coding region, such as -9 kb and -6 kb (etc.), are relative to the ATG start translation codon. The reporter gene constructs pGL3-PSCA (9 kb), pGL3-PSCA (6 kb), pGL3-PSCA (3 kb), and pGL3-PSCA (1 kb) were operatively linked to the luciferase gene (Figure 42). Plasmid, pGL3-CMV contains the cytomegalovirus promoter (Boshart, M. et al., 1985 *Cell* 41:521-530) linked to the luciferase gene and was used as a positive control. Also, plasmid pGL3 contains no promoter sequence and was used as a negative control plasmid.

Please replace the paragraph at page 127, lines 20-31, and continuing to page 128, line 2 with the following rewritten paragraph:

-- The nucleotide sequences of the genes encoding the heavy chain variable regions of murine monoclonal antibodies 1G8, 4A10 and 2H9 were determined using the methods described in Coloma et al., 1992, *J Immunol. Methods* 153: 89-104. Primers for heavy chain variable region sequencing of mAbs 1G8 and 4A10 were as follows:

D8
 HLEAD.1: ggc gat atc cac cat ggR atg Sag ctg Kgt Mat Sct ctt (SEQ ID NO. 25)

CH3': agg gaa ttc aYc toc aca cac agg RRc cag tgg ata gac (SEQ ID NO. 26)

Primers for heavy chain variable region sequencing of mAb 2H9 were as follows: